

# Effects of carbon dioxide, temperature and ultraviolet-B radiation and their interactions on soybean (*Glycine max* L.) growth and development

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## Abstract

Genetic modifications of agronomic crops will likely be necessary to cope with global climate change. Projected changes in global climate include increasing atmospheric carbon dioxide concentration ( $[\text{CO}_2]$ ), temperatures (T) and ultraviolet-B (UV-B) radiation which have significant effects on plants, however, their interactions are not clearly known to date. In this study we tested the hypothesis that soybean genotypes differ in growth and physiology with exposure to treatments of  $[\text{CO}_2]$  [360 and 720  $\mu\text{mol mol}^{-1}$  ( $+\text{CO}_2$ )], temperature [30/22 and 38/30 °C ( $+T$ )] and UV-B radiation [0 and 10  $\text{kJ m}^{-2} \text{d}^{-1}$  ( $+\text{UV-B}$ )] and their interactions. Six soybean genotypes (D 88-5320, D 90-9216, Stalwart III, PI 471938, DG 5630 RR, and DP 4933 RR) representing five maturity groups were grown in eight sunlit, controlled environment chambers in which control treatment had 360  $\mu\text{mol mol}^{-1}$   $[\text{CO}_2]$  at 30/22 °C temperatures and 0  $\text{kJ UV-B}$ . Results showed that elevated  $\text{CO}_2$  levels compensated the damaging effects caused by negative stressors such as high temperature and high UV-B radiation levels on most of the growth and physiological parameters studied. Total stress response index (TSRI) for each genotype was developed from the cumulative sum of response indices of vegetative and physiological parameters such as plant height, leaf area, total biomass, net photosynthesis, total chlorophyll content, phenolic content, relative injury and wax content. Based on TSRI, the genotypes were classified as tolerant (PI 471938 and D 88-5320), intermediate (DG 5630 RR and D 90-9216) and sensitive (DP 4933 RR and Stalwart III). The disruption of growth and physiology was significantly reduced in tolerant genotypes compared to sensitive genotypes. Strong correlations between total response of relative injury (RI), an indicator of cell membrane thermo stability and TSRI developed in this study show that RI could be used to predict the overall vegetative performance of the crop. However, the total response of RI did not show any linear correlation with TSRI of our previous study (which was developed with responses of reproductive traits). This suggests that there is a need to develop better screening tools and/or breeding strategies in developing genotypes suitable to cope future climates at both vegetative and reproductive stages.

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**Keywords:** Carbon dioxide; Growth; Physiology; Soybean; Temperature; Ultraviolet-B radiation

## 1. Introduction

First domesticated in Northeast parts of China, soybean has been adapted and commercially cultivated in all climatic zones of USA. Current  $[\text{CO}_2]$  levels of 360  $\mu\text{mol mol}^{-1}$  would reach about 560 and 970  $\mu\text{mol mol}^{-1}$  by middle or later part of the 21st century (Houghton et al., 2001). Since industrialization, the Earth's surface temperature has increased by 0.6 °C mainly due to changes in  $[\text{CO}_2]$  and other greenhouse gases during that period (Stott et al., 2000). Future changes in the greenhouse

gases are projected to increase surface temperature by another 1.5–4.5 °C by the end of this century (Houghton et al., 2001). Adding to these factors, reductions in the ozone column have led to substantial increases in UV-B radiation at the Earth's surface with the amount and intensity dependent on atmospheric and geographic factors (Madronich et al., 1998). The UV-B levels were 4–9  $\text{kJ m}^{-2} \text{d}^{-1}$  during June–August 2002 over USA soybean growing areas (<http://uvb.nrel.colostate.edu/UVB/>) and are projected to increase by 14–40% in near future. The relative plasticity of soybean growth and development to the projected abiotic factors will play a major role in determining the crops future.

The complex nature of soybean response to either single stress factor or interaction effects of two stress factors

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(CO<sub>2</sub> × temperature and CO<sub>2</sub> × UV-B) under projected climate scenarios has been well established. Increased photosynthesis and associated changes in morphology in response to elevated [CO<sub>2</sub>] increased soybean yield by 24–37% (Allen and Boote, 2000; Ainsworth et al., 2002). In contrast, high temperatures can inhibit photosynthetic carbon assimilation (Berry and Bjorkman, 1980) due to which some of the benefits of elevated CO<sub>2</sub> to plants may not be fully realized (Morison and Lawlor, 1999). Recent analysis of long term data revealed that growing season temperatures had negative impact on soybean yields and caused 17% reduction for every 1 °C rise (Lobell and Asner, 2003). Previous modeling studies also suggest that the observed sensitivity to high temperatures is higher than previously expected (Brown and Rosenberg, 1997; Carbone et al., 2003). In contrast, CO<sub>2</sub> induced increases in seed yield and total plant biomass were maintained or increased within the elevated CO<sub>2</sub> and UV-B environment in soybean (Teramura et al., 1990). Variability in soybean genotypic tolerance to these individual stresses has also been well established (CO<sub>2</sub>, Ziska et al., 1998; Ziska and Bunce, 2000; high temperature, Sapers and Anaele, 1991; UV-B radiation, Feng et al., 2000).

Sunlit, controlled environment chambers have been used to examine the effects and interactions of these climate change factors on plants (Pickering et al., 1994) which showed alterations both in physiology and growth and development in many crops such as soybean (Allen and Boote, 2000), cotton (Reddy et al., 2004) and many other crops. Interaction studies showed that CO<sub>2</sub> did not alleviate the negative effects of high temperatures in dry bean (Prasad et al., 2003) and in cotton (Reddy et al., 2005); high UV-B levels in cotton (Zhao et al., 2003); and both high temperature and high UV-B levels in soybean reproductive parameters (Koti et al., 2005). Injury to reproductive organs like flower and pollen morphology which resulted in reduced pollen germination and pollen tube lengths under future climate conditions with high temperatures and high UV-B radiation levels in soybean was observed previously even under elevated [CO<sub>2</sub>] conditions (Koti et al., 2005). However, no study ever reported interaction effects of all these three environmental factors on growth and development of any crop.

Scenarios for future climate change will include all of the above-discussed factors and understanding soybean responses to these multiple climate stress factors is needed to develop suitable management and cultural practices for the future climates. Most of the abiotic stresses, whether alone or in combination with other factors at any stage of the plants life cycle can result in loss of yield due to effects on plant processes. Whilst the main effects of either increasing [CO<sub>2</sub>], or temperature and UV-B radiation on physiology, morphology, growth and biomass have been investigated extensively, none in which interactions of all these factors were studied. Vyn and Hooker (2002) suggested that the impact of an individual stress on corn growth depends on the intensity and possible interactions among other stress factors.

Screening soybean genotypes during vegetative phase for single abiotic stress has been found useful in selecting genotypes with higher yields. Differences in soybean genotype tolerance to

dark chilling were identified by analyzing photosynthesis during the vegetative phase (van Heerden et al., 2003) and cell membrane thermostability (CMTS) during vegetative stage, and these parameters have been suggested as indirect screening techniques for high temperature tolerance (Sullivan, 1972). It was also reported that CMTS measured in the leaves during reproductive stage is associated with reproductive attributes that are known to be injured with high temperatures in plants (Ismail and Hall, 1999). In contrast, Pantuwan et al. (2004) suggested using rice genotypes that screening for drought tolerance was less efficient during vegetative phase than during reproductive phase with grain yield as a selection criterion. Hence, knowledge of vegetative responses should aid in ascertaining how environmental factors affect the growth and physiology of soybean plants and their use in determining reproductive tolerance under multiple stress conditions.

With this background, we hypothesize that magnitude and direction of genotypic tolerance to each of the three stressors will be modified by the interactive effect of these stressors and that vegetative parameters can be used to identify tolerance during reproduction. Our objectives were: (1) to understand the interaction effects of CO<sub>2</sub>, temperature and UV-B radiation on growth and physiology of soybean; (2) to classify genotypes based on their tolerance to these stressors and their interaction; (3) to understand the relationship between performance of the crop at both vegetative and reproductive stages.

## 2. Materials and methods

### 2.1. Experimental conditions, cultivars and plant husbandry

Soil-Plant-Atmosphere-Research (SPAR) chambers located at the R.R. Foil Plant Science Research facility of Mississippi State University (33°28'N, 88°47'W), Mississippi, USA were used for this study. More details of operation and control of SPAR chambers have been described by Reddy et al. (1992, 2001).

Soybean genotypes, Delsoy (D) 88-5320 (maturity group VI, non-glyphosate-tolerant), and D 90-9216 (maturity group VII, non-glyphosate-tolerant), Stalwart III (maturity group III, non-glyphosate-tolerant), Plant Introduction (PI) 471938 (maturity group V, non-glyphosate-tolerant), Deltagrow (DG) 5630RR (maturity group V, glyphosate-tolerant), and Deltapine (DP) 4933RR (maturity group IV, glyphosate-tolerant) were sown on 5 August 2003 in pots filled with fine sand. Thirty 2.5 L pots (five pots for each genotype) were arranged randomly in each SPAR chamber. Plants were watered three times a day with half-strength Hoagland's nutrient solution (Hewitt, 1952), delivered at 0800, 1200 and 1600 h, to ensure favorable nutrient and water conditions for plant growth through an automated and computer-controlled drip system. Variable-density black shade cloth around the edges of plants were adjusted regularly to match plant height in order to simulate natural shading caused by presence of other plants.

## 2.2. Treatments

The treatments included combinations of two CO<sub>2</sub> levels [360 and 720  $\mu\text{mol mol}^{-1}$  (+CO<sub>2</sub>)], two levels of temperature [30/22 and 38/30 °C (+T)] and two daily biologically effective UV-B radiation intensities of 0 and 10 [(+UV-B)]  $\text{kJ m}^{-2} \text{d}^{-1}$ . Control treatment is of 30/22 °C, 360  $\mu\text{mol mol}^{-1}$  [CO<sub>2</sub>] and 0  $\text{kJ m}^{-2} \text{d}^{-1}$  UV-B treatments. All SPAR chambers were maintained at 30/22 °C and at 360  $\mu\text{mol mol}^{-1}$  [CO<sub>2</sub>] until 10 days after sowing (DAS). Thereafter each chamber was set at one of the eight treatments. Air temperature in each SPAR chamber was monitored and adjusted every 10 s throughout the day and night and maintained within  $\pm 0.5$  °C of the treatment set points measured with shielded, aspirated thermocouples. The daytime temperature was initiated at sunrise and returned to the nighttime temperature 1 h after sunset. Constant humidity was maintained by operating solenoid valves that injected chilled mixture of ethylene glycol and water through the cooling coils located outside the air handler of each chamber. These cooling coils condensed excess water vapor from the chamber air in order to regulate relative humidity at 60% (McKinion and Hodges, 1985).

The chamber [CO<sub>2</sub>] was measured with a dedicated infrared gas analyzer (LI-COR, model LI-6252, Lincoln, Nebraska, USA) from the gas sample that is drawn through the lines run underground from SPAR units to the field laboratory building. Moisture is removed from the gas sample by running the sample lines through refrigerated water (4 °C) that was automatically drained and through a column of magnesium perchlorate. Chamber [CO<sub>2</sub>] is maintained by supplying pure CO<sub>2</sub> from a compressed gas cylinder through a system that included a pressure regulator, solenoid and needle valves and a calibrated flow meter (Reddy et al., 2001).

The desired UV-B radiation was supplied by square-wave UV-B supplementation systems under near ambient PAR. The UV-B radiation was delivered to plants for eight hours, each day, from 08:00 to 16:00 h by eight fluorescent UV-313 lamps (Q-Panel Company, Cleveland, Ohio, USA) powered by 40 W variable dimming ballasts. The lamps were wrapped with presolarized 0.07 mm cellulose diacetate film to filter UV-C (<280 nm) radiation. The cellulose diacetate film was changed at 3–4-d intervals. The UV-B energy delivered at the top of the plant canopy was checked daily at 09:00 h with a UVX digital radiometer (UVP Inc., San Gabriel, California, USA) calibrated against an Optronic Laboratory (Orlando, Florida, USA) Model 754 Spectroradiometer, which was used initially to quantify lamp output. The lamp output was adjusted, as needed, to maintain the respective UV-B radiation levels. A distance of 0.5 m from lamps to the top of plants was always maintained throughout the experiment. The actual biologically effective UV-B radiation was measured during the crop growth period at six different locations in each SPAR unit corresponding to the pots arranged in rows. The weighted total biologically effective UV-B radiation levels received at the top of the plants were  $9.8 \pm 0.16$  for +UV-B,  $9.6 \pm 0.07$  for +T + UV-B,  $9.6 \pm 0.07$  for +CO<sub>2</sub> + UV-B, and  $9.5 \pm 0.10$   $\text{kJ m}^{-2} \text{d}^{-1}$  for +CO<sub>2</sub> + T + UV-B treatments using the generalized plant response action spectrum normalized at 300 nm (Caldwell, 1971).

## 2.3. Measurements

**Phenology and growth:** Nodes were counted and plant heights were measured on all the plants at final harvest (51 DAE), few days after flowering. Leaf area was measured using the LI-3100 leaf area meter (LI-COR). Plant component dry weights were measured from all plants after oven drying at 75 °C until it weighed constant during a period of 72 h.

**Photosynthesis:** Leaf photosynthesis (Pn), on each of the selected leaves, fourth or fifth from the terminal from three different plants from all the units were measured using infrared gas analyzers built into a leaf cuvette in an open-gas exchange system (Li-Cor 6400; Li-Cor Inc., Lincoln, NE, USA). All measurements were made using a red-blue light source (LI-6400-02B) and adjusted to provide a fixed photosynthetic photon flux density of 1500  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  at 39 DAS. Cuvette block temperature was maintained to match the treatment daytime temperature using a computer-controlled peltier model mounted on the cuvette. Relative humidity inside the cuvette was maintained at approximately 40%. The [CO<sub>2</sub>] of airflow entering the cuvette was maintained at the treatment set points.

**Leaf pigments:** Photosynthetic pigments were extracted from the leaves used for photosynthetic measurements by placing five 38.5 mm<sup>2</sup> leaf discs in a vial with 5 mL of dimethyl sulfoxide and extracting for 24 h in dark at 42 DAS. The absorption of the extracts was determined at 470, 648 and 664 nm, using the Bio-Rad UV-vis spectrophotometer with a resolution of 1 nm by scanning from 200 to 900 nm. The equations of Lichtenthaler (1987) were used to estimate chlorophyll a and chlorophyll b concentrations. From these estimates, total chlorophyll content was calculated and expressed on a leaf area basis ( $\mu\text{g cm}^{-2}$ ).

**UV-B absorbing compounds:** The UV-B absorbing compounds were extracted from five 38.5 mm<sup>2</sup> leaf discs placed in a vial with 10 mL of extractant, a mixture of methanol, water, and hydrochloric acid in 79:20:1 ratio (Mirecki and Teramura, 1984) at 42 DAS. The vials were incubated at room temperature for 24 h in dark to allow for complete extraction of UV-B absorbing compounds. The absorbance of these extracts from different treatments was measured at 300 nm (Kakani et al., 2004). The content of UV-B absorbing compounds was calculated using the equation;  $y = 16.05 \times A$ , where y is concentration of UV-B absorbing compounds ( $\mu\text{g mL}^{-1}$  of extract) expressed as equivalents of p-coumaric acid and A is absorbance at 300 nm.

**Relative injury (RI):** The technique we used to determine RI was similar to that developed by Martineau et al. (1979) with minor modifications. Fully expanded leaves were collected from three different plants from all the units of all the genotypes at 42 DAS. Two sets (control, C and treatment, T) of 2.5 cm<sup>2</sup> leaf discs from approximately five randomly selected leaves were placed in the test tubes containing 10 mL of de-ionised water. The leaf segments were thoroughly rinsed with three changes of de-ionised water to remove electrolytes adhering to, as well as electrolytes released from the cut surface of the segments. After final rinsing, the T set of tubes were drained, capped with aluminum foil to prevent dehydration of tissue during heat treat-

ment and incubated in a hot water bath at 50 °C for 20 min, while the C set of tubes were kept at 25 °C. After incubation, T sets of tubes were brought to 25 °C and then both C and T sets of tubes were incubated at 10 °C for 18 h. After they were brought to 25 °C, conductance was measured in C (CEC1) and T (TEC1) set of tubes. Tubes were then placed in an autoclave at 0.1 MPa for 12 min to completely kill the tissue, releasing all the electrolytes. Tubes were then cooled to 25 °C and then final conductance was measured in both C (CEC2) and T (TEC2) test tubes. The relative injury (RI) to the tissues was measured as following Martineau et al. (1979):

$$RI (\%) : 1 - \frac{1 - (TEC1/TEC2)}{1 - (CEC1/CEC2)} \times 100$$

**Wax content:** The uppermost fully expanded leaves (fourth or fifth leaves from the top of the mainstem) of three different plants from all the units were used to determine the epicuticular wax content at 42 DAE. Each leaf was washed with 20 mL of chloroform for 20 s and the chloroform poured into test tubes whose initial weights were recorded. The chloroform in the tubes was evaporated with an evaporator and the final weights of the test tubes were recorded. The wax content was calculated by subtracting initial weight of the tube from the final weight and expressed as  $\mu\text{g mm}^2$ .

#### 2.4. Cumulative stress response index (CSRI)

The cumulative stress response index (CSRI) was calculated as the sum of relative individual component responses at each treatment and is similar to the combined response index quoted in other UV-B studies (Dai et al., 1994; Koti et al., 2005). CSRI was calculated to evaluate the vegetative and physiological responses of soybean to the treatments under study (+CO<sub>2</sub>, +T, +CO<sub>2</sub> + T, +UV-B, +CO<sub>2</sub> + UV-B, +CO<sub>2</sub> + T, and +CO<sub>2</sub> + T + UV-B) in comparison to control treatment. The

CSRI was calculated as follows (Dai et al., 1994).

$$CSRI = \left( \frac{PH_t - PH_c}{PH_c} + \frac{LA_t - LA_c}{LA_c} + \frac{TB_t - TB_c}{TB_c} + \frac{Pn_t - Pn_c}{Pn_c} + \frac{TC_t - TC_c}{TC_c} + \frac{Phe_t - Phe_c}{Phe_c} + \frac{RI_t - RI_c}{RI_c} + \frac{Wx_t - Wx_c}{Wx_c} \right) \times 100$$

where CSRI is the cumulative stress response index, PH the plant height, LA the leaf area of the plant, TB the total biomass of plant, Pn the net photosynthesis, TC the total chlorophyll content, Phe the phenolic content, RI the relative injury and Wx is the wax content under t (treatment) and c (control). Genotypes were classified into tolerant, intermediate and sensitive based on total stress response index (TSRI), the sum of CSRI over all the treatments.

#### 2.5. Statistical analysis

Statistical analysis was conducted by using two-way analysis of variance (ANOVA) (SAS Institute Inc., 1997). The least significant difference tests at  $P = 0.05$  were used to distinguish treatment differences for the growth and physiological parameters in the study. The standard errors of each mean were calculated and presented in the graphs as error bars. Genotypes were classified based on TSRI-vegetative of all the treatments as tolerant [ $>\text{minimum TSRI} + 2 \text{ standard deviation (stdev)}$ ], intermediate [ $>\text{minimum TSRI} + 1 \text{ stdev}$  and  $<\text{minimum TSRI} + 2 \text{ stdev}$ ] and sensitive [ $<\text{minimum TSRI} + 1 \text{ stdev}$ ].

### 3. Results

**Plant height:** Significant CO<sub>2</sub> × T ( $P < 0.001$ ), T × UV-B ( $P < 0.01$ ) and CO<sub>2</sub> × T × UV-B ( $P < 0.001$ ) interactions were

Table 1  
The analysis of variance across the treatments of carbon dioxide concentrations ([CO<sub>2</sub>]), temperature (T), UV-B radiation (UV-B) and genotype (G) and their interactions on soybean growth and physiology

Source of variation	Plant height <sup>a</sup>	Leaf area	Total biomass	Photosynthesis	Phenolic content	Total chlorophyll	Wax content	Relative injury
Carbon dioxide ([CO <sub>2</sub> ])	***	***	***	***	*	NS	**	***
Temperature (T)	***	***	***	***	***	NS	NS	***
[CO <sub>2</sub> ] × T	***	***	***	NS	**	NS	**	**
UV-B radiation (UV-B)	***	***	***	***	***	***	**	***
[CO <sub>2</sub> ] × UV-B	**	NS	NS	NS	***	**	NS	***
T × UV-B	**	*	***	***	NS	NS	*	***
[CO <sub>2</sub> ] × T × UV-B	***	***	***	***	NS	**	*	NS
Genotype (G)	***	NS	**	NS	NS	NS0	NS	**
[CO <sub>2</sub> ] × G	***	**	NS	***	*	**	NS	NS
T × G	***	NS	*	***	NS	*	**	*
UV-B × G	NS	NS	NS	***	**	NS	NS	***
[CO <sub>2</sub> ] × T × G	NS	NS	NS	*	NS	NS	NS	NS
[CO <sub>2</sub> ] × UV-B × G	*	NS	NS	NS	NS	NS	NS	NS
T × UV-B × G	***	NS	NS	***	NS	NS	*	NS
[CO <sub>2</sub> ] × T × UV-B × G	NS	NS	NS	*	NS	NS	*	NS

Significance levels are indicated by \*\*\*, \*\*, \* and NS representing  $P < 0.001$ ,  $P < 0.01$ ,  $P < 0.05$  and  $P > 0.05$ , respectively.

<sup>a</sup> The observations of plant height, leaf area and total biomass were recorded at 51 DAS; photosynthesis measurements at 39 DAS and all other physiological measurements at 42 DAS.



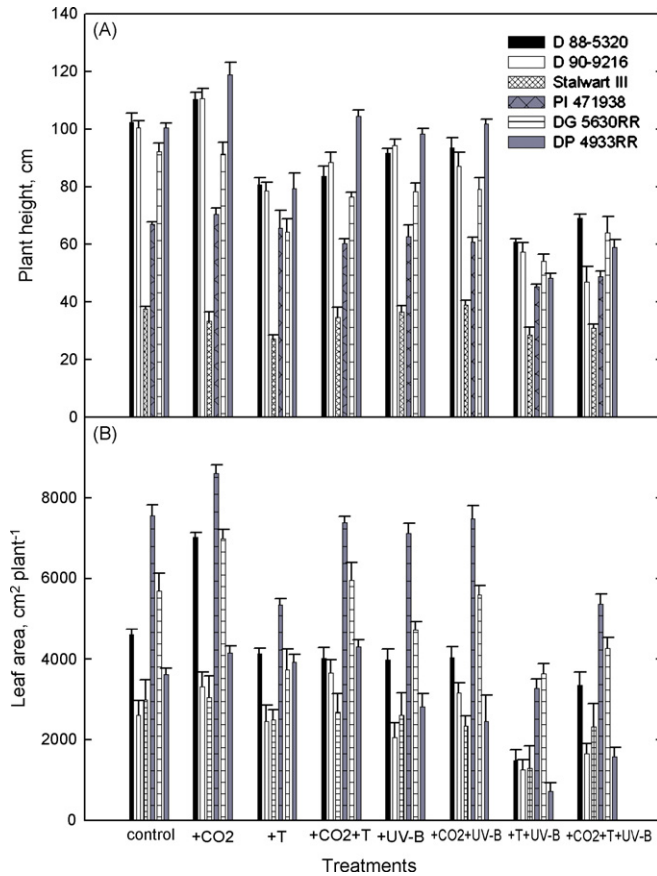


Fig. 1. Influence of carbon dioxide concentration (control,  $360 \mu\text{mol mol}^{-1}$  and  $+[\text{CO}_2]$ ,  $720 \mu\text{mol mol}^{-1}$ ), temperature (control,  $30/22^\circ\text{C}$  and  $+T$ ,  $38/30^\circ\text{C}$ ), UV-B radiation (control, 0 and  $+UV-B$ ,  $10 \text{ kJ m}^{-2} \text{ d}^{-1}$ ), and their interactions on (A) plant height and (B) leaf area for six soybean genotypes. Bars represent standard errors ( $n=5$ ).

observed for plant height. Taller plants were observed when plants were grown under control or  $+CO_2$  conditions. Plants grown under  $+T$  conditions were shorter either alone (21%) or in combination with  $+CO_2$  (10%). While  $+UV-B$  alone or along with  $+CO_2$  had minimal negative effects on plant height (7%), but when grown along with  $+T$ , the plant heights were 34% less when averaged over all the genotypes. At  $+CO_2 + T + UV-B$  conditions, the greatest reduction in plant height (53%) was observed in D 90-9216 while the reduction was only 18% in Stalwart III compared to the plants grown in control conditions (Table 1 and Fig. 1A).

**Leaf area:** Plants grown under  $+CO_2$  conditions had significantly more leaf area than control plants (Table 1 and Fig. 1B) over all the genotypes, while temperature and UV-B had a negative effect on plants either alone (−15%) or together (−58%) when compared to the plants grown under control conditions. But, in plants grown under  $+CO_2 + T$  and  $+CO_2 + UV-B$ , the reductions were not as large as the reductions observed under single stress conditions. When the plants were subjected to all the stressors ( $+CO_2 + T + UV-B$ ), the leaf areas were 22% (Stalwart III) to 56% (DP 4933RR) less than the plants that are grown under control conditions.

**Total biomass:** Similar to leaf area responses, total biomass responded to various stress conditions. The  $+CO_2$  conditions

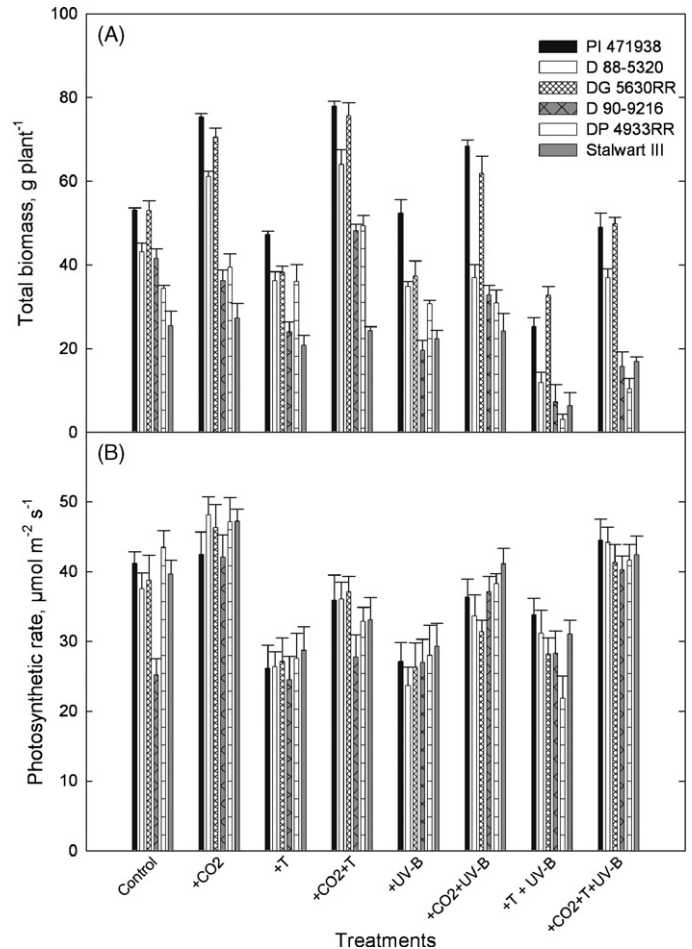


Fig. 2. Influence of carbon dioxide concentration (control,  $360 \mu\text{mol mol}^{-1}$  and  $+[\text{CO}_2]$ ,  $720 \mu\text{mol mol}^{-1}$ ), temperature (control,  $30/22^\circ\text{C}$  and  $+T$ ,  $38/30^\circ\text{C}$ ), UV-B radiation (control, 0 and  $+UV-B$ ,  $10 \text{ kJ m}^{-2} \text{ d}^{-1}$ ), and their interactions on (A) total biomass and (B) photosynthetic rate of six soybean. Bars represent standard errors ( $n=5$  for biomass and  $n=3$  for photosynthetic rate).

alone significantly increased total biomass by 21%, averaged over all the genotypes, D 88-5320 had the highest positive response (42%) (Table 1 and Fig. 2A) in comparison to control conditions. While, plants grown under high temperatures and enhanced UV-B radiation had less total biomass, by 18 and 21%, respectively. But under  $+T + CO_2$  and  $+CO_2 + UV-B$  conditions, the reductions in biomass were not evident. Plants grown under  $+T + UV-B$  had the significantly less total biomass (69%) than plants grown under  $+CO_2 + T + UV-B$  conditions, and when averaged over all the genotypes, the reductions ranged from 38% (DG 5630RR) to 91% (DP 4933RR).

**Photosynthetic rate:**  $+CO_2$  conditions had a significant positive effect on photosynthetic rates in most of the genotypes while D 90-9216 had the highest positive response (42%). Significant negative effect on photosynthetic rates was observed with high temperatures (27%) and enhanced UV-B levels (26%) when grown under single stress conditions, but in combination, the reductions were 20%. The  $T \times UV-B$  and  $CO_2 \times T \times UV-B$  interactions were significant ( $P < 0.001$ ). Genotype D 90-9216 had significantly less photosynthetic rates when averaged across treatments (Table 1 and Fig. 2B).

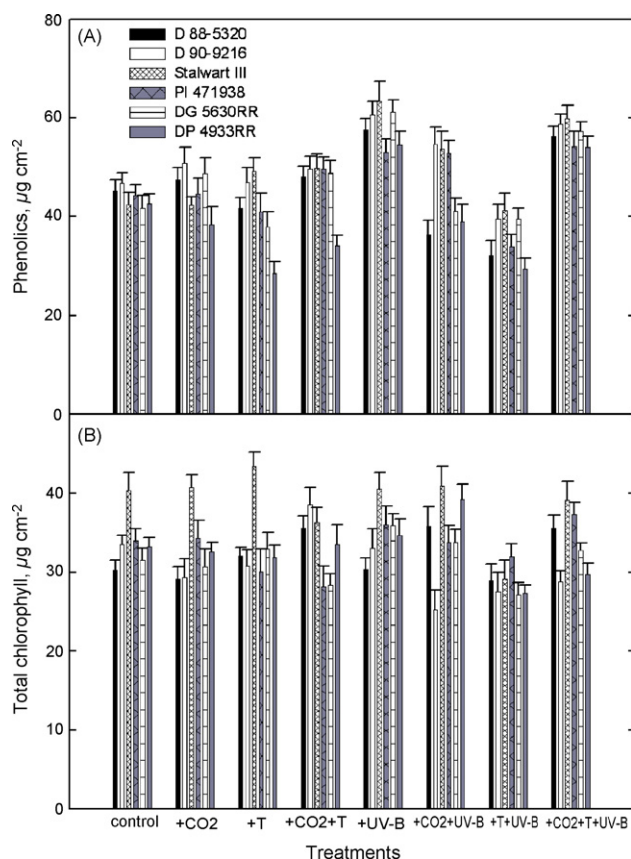


Fig. 3. Influence of carbon dioxide concentration (control,  $360 \mu\text{mol mol}^{-1}$  and +[CO<sub>2</sub>],  $720 \mu\text{mol mol}^{-1}$ ), temperature (control,  $30/22^\circ\text{C}$  and +T,  $38/30^\circ\text{C}$ ), UV-B radiation (control, 0 and +UV-B,  $10 \text{ kJ m}^{-2} \text{ d}^{-1}$ ), and their interactions on (A) phenolics and (B) total chlorophyll content of six soybean genotypes. Bars represent standard errors ( $n=3$ ).

**Phenolic content:** Significant CO<sub>2</sub>  $\times$  T and CO<sub>2</sub>  $\times$  UV-B interactions were observed for leaf phenolic concentrations of soybean plants (Table 1). Averaged over all the genotypes, high temperatures decreased the phenolic content by 14%, +UV-B conditions increased by 21% with highest increase in DG 5630RR (46%) and lowest increase in D 90-9216 (6%), and no difference in phenolic content was observed for PI 471938. Under +CO<sub>2</sub> + T conditions, the reductions were not observed while with +T + UV-B conditions, the reductions ranged from 5 to 37%. Compared to the control conditions, +CO<sub>2</sub> + T + UV-B conditions increased the phenolic concentrations in D 88-5320, D 90-9216 and DG 5630RR, decreased in DP 4933RR and PI 471938 and no effect was observed in Stalwart III (Fig. 3A).

**Total chlorophyll content:** Significant UV-B and CO<sub>2</sub>  $\times$  UV-B effects were observed for total chlorophyll content (Table 1). In some genotypes such as DG 5630RR and PI 471938 there was 6–14% increase in total chlorophyll content for plants grown in +UV-B conditions. Where as at +CO<sub>2</sub> + T + UV-B conditions, there was 18% increase in total chlorophyll content in D 88-5320 and DP 4933RR while in D 90-9216, +CO<sub>2</sub> + T + UV-B conditions reduced the total chlorophyll content by 25%. On an average, +T + UV-B conditions decreased the total chlorophyll content of all the genotypes (15%) with highest reductions occur-

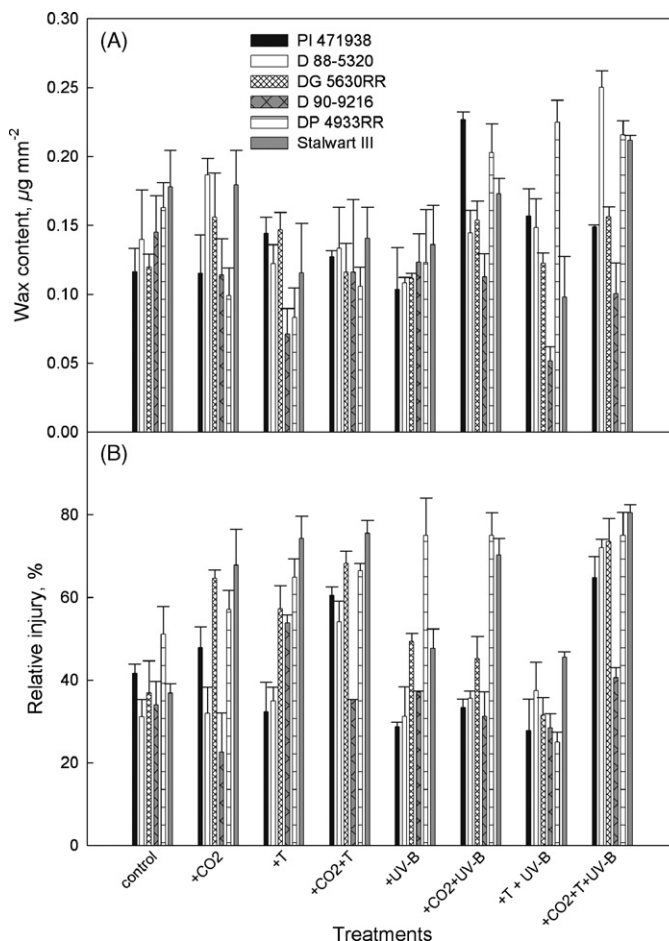


Fig. 4. Influence of carbon dioxide concentration (control,  $360 \mu\text{mol mol}^{-1}$  and +[CO<sub>2</sub>],  $720 \mu\text{mol mol}^{-1}$ ), temperature (control,  $30/22^\circ\text{C}$  and +T,  $38/30^\circ\text{C}$ ), UV-B radiation (control, 0 and +UV-B  $10 \text{ kJ m}^{-2} \text{ d}^{-1}$ ), and their interactions on (A) wax content and (B) relative injury of the membranes of six soybean genotypes. Bars represent standard errors ( $n=3$ ).

ring in Stalwart III (28%) and lowest reductions in D 88-5320 (5%) (Fig. 3B).

**Wax content:** Significant CO<sub>2</sub>  $\times$  T, T  $\times$  UV-B and CO<sub>2</sub>  $\times$  T  $\times$  UV-B interactions were observed for wax content (Table 1). The wax content ranged from 0.11 to  $0.18 \mu\text{g mm}^{-2}$  in the plants and  $0.10\text{--}0.25 \mu\text{g mm}^{-2}$  in the +CO<sub>2</sub> + T + UV-B conditions (Fig. 4A). Plant grown in +T + UV-B conditions exhibited more (21–26%) wax on leaves when compared to the wax content of leaves of plants grown in control conditions.

**Relative injury of the membranes:** Significant interactions (CO<sub>2</sub>  $\times$  T, CO<sub>2</sub>  $\times$  UV-B and T  $\times$  UV-B) were observed for relative injury of the membranes (Table 1). Stalwart III and DG 5630RR had more relative injury to the membranes with +CO<sub>2</sub>, while in the other genotypes, +CO<sub>2</sub> conditions did not cause any injury to the membranes (Fig. 4B). In Stalwart III and DG 5630RR, +T and +CO<sub>2</sub> + T + UV-B conditions had a large effect on membranes, and the relative injury of membranes increased by 100 and 55% in +T and 81 and 75% in +CO<sub>2</sub> + T + UV-B treatments, respectively. On an average over all the genotypes, there was 59% more relative injury at +CO<sub>2</sub> + T treatments (Fig. 4B).

Table 2

Cumulative stress response index (CSRI), sum of relative individual component responses at a given treatment; and total stress response index (TSRI), pooled over all the treatments of six soybean genotypes in response to elevated carbon dioxide ( $720 \mu\text{mol mol}^{-1}$ , +[CO<sub>2</sub>]), high temperature ( $38/30^\circ\text{C}$ , +T) and increased UV-B radiation ( $10 \text{ kJ m}^{-2} \text{ d}^{-1}$ , +UV-B) and their interactions

Treatments	Genotypes					
	PI 471938	D 88-5320	DG 5630RR	D 90-9216	DP 4933RR	Stalwart III
Cumulative stress response index (CSRI)						
+ [CO <sub>2</sub> ]	+53.9 (4)	+163.2 (1)	+75.2 (3)	+82.9 (2)	−6.6 (5)	−31.1 (6)
+T	−74.9 (1)	−97.5 (2)	−136.9 (4)	−133.7 (3)	−158.4 (5)	−185.8 (6)
+ [CO <sub>2</sub> ] + T	−26.9 (4)	−0.6 (2)	−19.2 (3)	+70.7 (1)	−43.4 (5)	−138.7 (6)
+UV-B	−33.1 (1)	−75.5 (3)	−59.5 (2)	−88.2 (5)	−111.4 (6)	−76.4 (4)
+ [CO <sub>2</sub> ] + UV-B	+112.9 (1)	−50.5 (4)	+3.2 (3)	+8.4 (2)	−68.1 (6)	−73.3 (5)
+T + UV-B	−144.2 (1)	−234.5 (3)	−151.5 (2)	−255.1 (5)	−246.8 (4)	−287.1 (6)
+ [CO <sub>2</sub> ] + T + UV-B	−67.2 (3)	+4.9 (1)	−65.6 (2)	−136.8 (5)	−213.5 (6)	−123.1 (4)
Total stress response index	−179.7 (1)	−290.5 (2)	−354.4 (3)	−451.8 (4)	−847.9 (5)	−915.5 (6)

TSRI is the sum of relative responses with treatments in comparison to control ( $360 \mu\text{mol mol}^{-1}$  [CO<sub>2</sub>],  $30/22^\circ\text{C}$  and  $0 \text{ kJ m}^{-2} \text{ d}^{-1}$ ) observed for vegetative and physiological parameters studied (plant height, leaf area, total biomass and net photosynthetic rate, total chlorophyll, phenolics, relative injury and wax concentrations). The numbers in parenthesis indicate the ranks of genotypes given in accordance to their performance in a particular environment.

**Total stress response index (TSRI):** TSRI is the sum of the individual cumulative stress response indices of all the treatments for all the vegetative and physiological parameters studied. Based on TSRI, the genotypes were classified as tolerant (D 88-5320 and PI 471938) (>minimum TSRI + 2 stdev: >−307), intermediate (DG 5630RR and D 90-9216) (>minimum TSRI + 1 stdev and < minimum TSRI + 2 stdev: >−611 and <−307) and sensitive (DP 4933RR and Stalwart III) (<minimum TSRI + 1 stdev: <−611) (Table 2). No significant linear correlation was observed between TSRI of vegetative and physiological parameters derived from this study and TSRI of reproductive parameters of our previous study (Koti et al., 2005) ( $R^2 = 0.56$ ) (Fig. 5). However, a significant linear correlation was observed when total response index of RI was plotted against TSRI of the present study ( $R^2 = 0.58$ ) and the relationship was not sig-

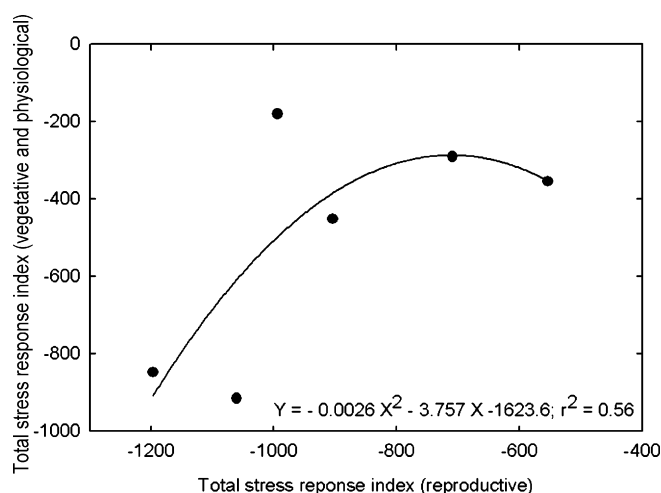


Fig. 5. Relationship between total stress response index (TSRI), calculated as a response of vegetative and physiological parameters with TSRI of reproductive parameters of six soybean genotypes grown under the influence of carbon dioxide concentration (control,  $360 \mu\text{mol mol}^{-1}$  and +[CO<sub>2</sub>],  $720 \mu\text{mol mol}^{-1}$ ), temperature (control,  $30/22^\circ\text{C}$  and +T,  $38/30^\circ\text{C}$ ), UV-B radiation (control, 0 and +UV-B,  $10 \text{ kJ m}^{-2} \text{ d}^{-1}$ ) treatments.

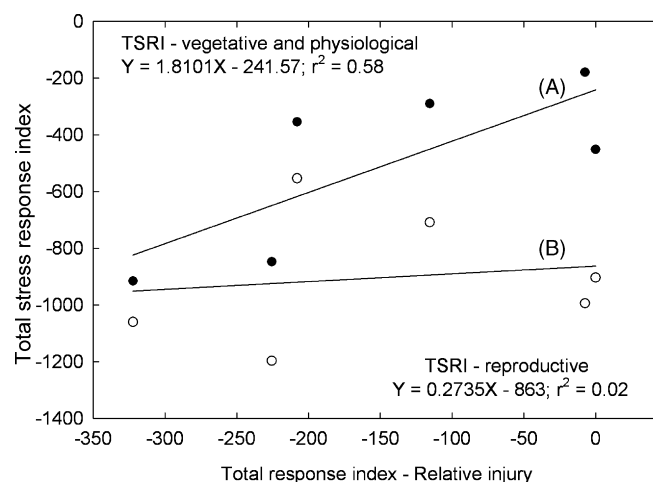


Fig. 6. Relationship between total stress response index (TSRI), calculated as a response of (A) vegetative and physiological parameters and (B) TSRI of reproductive parameters with total response index of relative injury of six soybean genotypes grown under the influence of carbon dioxide concentration (control,  $360 \mu\text{mol mol}^{-1}$  and +[CO<sub>2</sub>],  $720 \mu\text{mol mol}^{-1}$ ), temperature (control,  $30/22^\circ\text{C}$  and +T,  $38/30^\circ\text{C}$ ), UV-B radiation (control, 0 and +UV-B,  $10 \text{ kJ m}^{-2} \text{ d}^{-1}$ ) treatments.

nificant when plotted against TSRI of reproductive parameters ( $R^2 = 0.02$ ) (Fig. 6).

#### 4. Discussion

The results from the present study provide evidence for individual and interactive effects of three important environmental factors that are projected to change in the future climate on soybean growth and development. Our results showed that elevated [CO<sub>2</sub>] partially compensated the damaging effects on vegetative growth and physiology caused by negative stressors such as high temperatures and enhanced UV-B radiation levels in soybean. It was hypothesized that genotypic differences to a stressor will be modified by the interactive effect of multiple stressors. Sup-

porting our hypothesis, genotypic variation that was observed in most of the vegetative and physiological responses to the environmental factors studied was modified by their interactive effects. The genotypes responded in the same direction for the negative stressors (temperature and UV-B), however, the magnitude of their response varied significantly.

The identified tolerant genotypes (D 88-5320 and PI 471938) had less leaf area and total biomass reductions due to the imposed negative stressors (high temperatures and high UV-B levels) when compared to the sensitive genotypes. Plants heights, on the other hand, had no distinct pattern with stress effects. The effect of UV-B on plant heights, however, was significant while, [CO<sub>2</sub>] or temperature effects were not. Similar to our findings, a recent data analysis by Anthor (2001) found that warming by only a few degrees may offset the positive effect of elevated CO<sub>2</sub> on crop yields. Although the leaf area was smaller under high UV-B conditions, plants showed higher chlorophyll content when expressed on leaf area basis. Similar responses to high temperatures of 36/28 °C were observed in cotton (Reddy et al., 2004) and UV-B levels in *Arabidopsis* (Jenkins et al., 1997). The reductions can be attributed to the breakdown of the structural integrity of chloroplasts (Cassi-Lit et al., 1997), which would have varied with genotypes. Alternations in phenotype can result in variations in biomass production among the genotypes (Caldwell and Flint, 1994). This can also be due to minimal reductions in photosynthesis in the identified tolerant genotypes. The observed increase in net photosynthesis under elevated CO<sub>2</sub> and at ambient temperatures (Ziska and Bunce, 1994) was not evident under ambient CO<sub>2</sub> and high temperature conditions. The stress dependent decrease of photosynthesis may be attributed to the lower enzyme activities of ribulose biphosphate carboxylase and phosphoenol pyruvate carboxylase and/or lower photosystem activity (Tevini and Pfister, 1985). Our observations are in contrast to the hypothesis of Long (1991) that increasing temperature would increase the affinity of ribulose biphosphate carboxylase/oxygenase for CO<sub>2</sub>, leading to an increase in the CO<sub>2</sub> stimulation of photosynthesis with temperature. Since net photosynthesis of the plants is reduced under high temperatures and high UV-B conditions, a lower supply of sugars for cell wall growth might also be involved in growth reductions.

One of the UV-B protective mechanisms that were previously reported in the literature was accumulation of UV-B absorbing compounds which absorbed mainly in the UV region of the spectrum and abound mainly in the epidermis (Grammatikopoulos et al., 1999). These compounds are considered to protect the underlying tissues against the harmful effects of solar UV-B radiation (Meijkamp et al., 1999). Levizou and Manetas (2002) showed significant correlations between total phenolic levels and UV-B absorbing capacity (simple methanolic absorbance at 300 nm). We observed genotypic variation in the production of these compounds at high UV-B levels, but we did not find any correlation between the TSRI and UV-B absorbing compounds, showing that even though UV-B absorbing compounds accumulated with the imposed stress but are not directly related to sensitivity/tolerance of soybean genotypes. Along with these compounds we also studied wax content of the plants, where

the wax layer acts as an interface between the environment and internal structures of leaf. Wax content increased in tolerant genotypes while it reduced in the susceptible genotypes suggesting its role in abiotic stress-tolerance. Wax layer is an important surface character that responds to environmental stresses such as water stress (Bondada et al., 1996), UV-B radiation (Kakani et al., 2004) and seasonal changes in temperature (Rao and Reddy, 1980). However, much information is not available to understand whether the effect was in response to stress or a protective mechanism.

In this study, we observed high correlation between total response index of RI and overall vegetative performance of the crop. Previously, in cowpea (Ismail and Hall, 1999) and in Kentucky bluegrass (Marcum, 1998), it was shown that measurements of CMTS have the potential to be used for screening for reproductive stage heat tolerance. Hall and Allen (1993) showed that cowpea cultivars with heat tolerance during reproductive development, and with high harvest index, high photosynthetic capacity per unit leaf area, small leaves and low leaf area per unit ground area under present levels of CO<sub>2</sub> will be most responsive to elevated CO<sub>2</sub> under both hot and intermediate temperatures. They showed that heat tolerance genes confer some of these hypothesized traits: heat tolerance during reproductive development, higher harvest index and less leaf area per unit ground area. Ahmed and Hall (1993) also showed that the heat tolerance genes in addition to enhancing grain yields under hot conditions also showed responses to elevated CO<sub>2</sub> with respect to pod production under both optimum and high temperatures. But, plants grown in the future climates will be experiencing increasing UV-B conditions along with increasing temperatures and CO<sub>2</sub> levels. This theory may not be effective if other stresses occur along with high temperatures. The observed positive correlations demonstrate a basis for the association of CMTS with overall stress-tolerance. However, CMTS having no linear correlation with reproductive performance of the plants strongly suggests the need for further studies. Such studies will lead to better screening tools or breeding strategies or genetic transformation technologies for developing genotypes suited to cope with global climate change.

One of our objectives was also to understand the sensitivity for vegetative and reproductive performance in response to these stresses. Previous reports also show differential sensitivity for reproductive and vegetative growth in soybean (Baker et al., 1989), and wheat (Mitchell et al., 1993) under high temperature conditions. The reproductive performance (pollen production, germination, pollen tube lengths and flower length) of the genotypes under the influence of these stresses was previously reported in the same genotypes (Koti et al., 2005). The correlation observed between vegetative performance (this study) and reproductive performance (Koti et al., 2005) shows that genotypes, which perform vegetatively well, do not respond in the same way for reproductive parameters. Arriving at better screening tools or breeding strategies for developing genotypes suitable to cope with abiotic stresses at both vegetative and reproductive stages is needed for crops to grow better under future climatic conditions.



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